AMINO ACIDS FROM FOSSILS, FACIES AND FINGERS

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ABSTRACT. The possibility of introducing contaminating amino acids while preparing fossils and rocks for analysis is a major problem for molecular palaeontology. The most important source of contamination is human finger tips, which source approximately 100 times the concentration of amino acid than New Zealand fossil shells. Latex gloves can also transmit appreciable quantities of modern day amino acids. However, hierarchical clustering and principal component analysis reveal that the relative proportions of finger-tip amino acids are constant to an extent which allows them to be readily distinguished from those for fossil shells and their enclosing sediment. This approach therefore represents a useful method of checking for contamination by finger tips in analyses of fossil molecules.

AMINO acids are ubiquitous in fossils and rocks, and represent a potentially rich source of information on a wide variety of geological topics (Schroeder and Bada 1976; Curry 1988). For palaeontology, there is considerable potential in studying amino acids from fossil shells and skeletons, for example in the determination of the isotopic composition of individual amino acids which were originally synthesized during the life of the fossil organism. The major problem, however, is that amino acids are so abundant and widely distributed in rocks, sediments and the atmosphere, that there is considerable potential for contamination of fossil shells by diffusion from surrounding sediments and rocks. It is partially with this consideration in mind that attention has recently focused on intracrystalline molecules from brachiopod shells, the rationale being that because such molecules are entombed within, rather than around, biocrystals (Collins *et al.* 1988), they were situated within a protective microenvironment where they were much less prone to contamination. Brachiopods contain a surprising number of different intracrystalline molecules, including various proteins which have recently been sequenced (Curry *et al.* 1991). It is the breakdown products of these proteins (i.e. amino acids and peptides) which offer the best opportunity of recovering genuinely indigeneous molecular information from fossils, and are present in all groups of fossils as far back as the Cambrian (Curry 1988).

However, concentrating on intracrystalline molecules does not completely solve the problem of contamination, because there is still the risk of introducing amino acids during sample preparation. Extraneous amino acids could be added to geological samples during storage (from bacteria, for example) or even by touching specimens with fingers, which are constantly shedding skin composed of vast numbers of amino acids. The question of contamination by finger-tip amino acids was first raised in 1965 (Hamilton 1965; Oró and Skewes 1965), but investigation of this phenomenon has not kept pace with technological advances in amino acid analysis, which have increased incrementally in sensitivity since this early, and often overlooked, work.

It was therefore essential to investigate both the abundance and the relative proportion of finger-tip amino acids as a prelude to a detailed investigation of the molecular palaeontology of fossil shells from New Zealand. It was hoped that identification and an understanding of the characteristics of such amino acids would, when compared with data generated from fossils and sediments using an identical high-sensitivity analysis system, allow the recognition of such contamination and hence eradicate it as a potential distortion of our analyses.

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GEOLOGICAL HISTORY OF SAMPLES

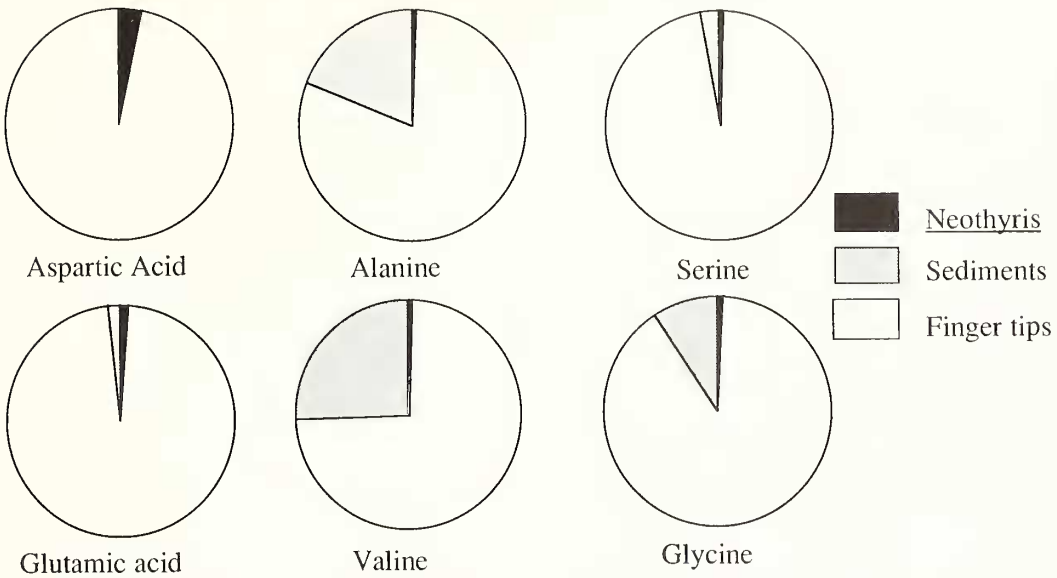
The mid-late Pleistocene sediments and brachiopod fossils investigated were collected from the South Wanganui Basin, which is one of a number of sedimentary basins in the south west of present day North Island, New Zealand. These basins were formed by tectonism related to the subduction of the Pacific Plate below North Island, causing maximum subsidence during the early Miocene. Since this time, there has been general tectonic emergence in these areas (Stern and Davey 1990), indicating that the major control of sedimentation during the mid-late Pleistocene were glacially moderated eustatic changes. Glaciation of South Island began during the Hautawan (*c.* 2·5 Ma) and caused repeated submergence/emergence of the sediments which, coupled with the continuing emergence of the region, resulted in a series of well preserved marine terraces, which have undergone very little burial, along the Taranaki coast (Fleming 1953). It is from these terraces that both the fossils and sediments were collected.

MATERIALS AND METHODS

Free amino acids from finger tips were collected from the fingers of members of the Department of Geology and Applied Geology, University of Glasgow. Sample selection was completely random with respect to the time of day, sex and age of the individuals. Citrate buffer, pH 2·2, was added to the base of a sterile Cel-cult tissue culture plate well (500 μl of 100 mM), and mixed on a Luckman (Model 802) suspension mixer to ensure complete coverage of the base. The index finger of each individual was wiped with a tissue immediately prior to sampling, to remove traces of solid particles, and then pressed into the well for 15 seconds. Nine samples were taken, including some from fingers which had been washed with either Millipore Reverse Osmosis Water or soap and tap water immediately prior to the test. One well was left untouched to provide a sample blank, and one was touched with the finger in a latex glove that had been used for sample preparation and collection. An aliquot (30 μl) of each of the samples was applied to the sample frit of the analysis system.

Surface contaminants were removed from a variety of fossil brachiopods by immersion of the shells in an aqueous solution of sodium hypochlorite (10% v/v), and scraping away the encrusting epifauna with a steel needle. Intercrystalline molecules from the powdered or disaggregated shells were destroyed by a second incubation with 10% (v/v) sodium hypochlorite. Amino acids were recovered by decalcifying the shells in an aqueous solution (20% w/v) of EDTA (Ethylene-diaminotriacetic acid, disodium salt) at pH 8. EDTA interferes with amino acid analysis, and was therefore removed prior to the analysis of the macromolecular components from the shell by dialysis or ultrafiltration using a 10 kDa cut-off membrane or filter. Amino acids were recovered from sediments by mixing 30 g of dry sediment with 50 ml of ultra-pure water (MilliQ™) in an airtight bottle held at 110 °C for 24 hours. When cool, the liquid was centrifuged (2 k.g.h.), filtered and finally concentrated using a rotary evaporator (Howe Gyrovap).

Hydrolysis of the peptide bonds between amino acids in a polypeptide is a prerequisite of amino acid analysis. Both manual and automatic vapour phase hydrolysis was employed in this study. For manual hydrolysis, 20 μl of the samples, in newly pyrolysed (500 °C/4 hours) glass hydrolysis tubes, were placed in an airtight flask with 500 μl of 6 N HCl, and heated to 165 °C for 60 minutes under an Argon blanket (Dupont *et al.* 1989). After hydrolysis the samples were reconstituted in an aqueous solution of EDTA (0·025% w/v; tripotassium salt) and thoroughly mixed. Automated hydrolysis, reproducing these conditions with 6N HCl vapour phase hydrolysis, was performed by the Applied Biosystems 420-H amino acid analyser. An aliquot (10–20 μl) of solution was loaded onto the amino acid analyser, and derivitized using PITC (Phenylisothiocyanate; Heinrikson and Meredith 1984), prior to identification using a linked narrow bore HPLC (High Performance Liquid Chromatography) system optimized for PTC-amino acids. Amino acids were identified and quantified relative to amino acid standards (Pierce standard H). All analyses were repeated at least once to ensure reproducibility. Water and buffers used were also analysed to check for contamination.



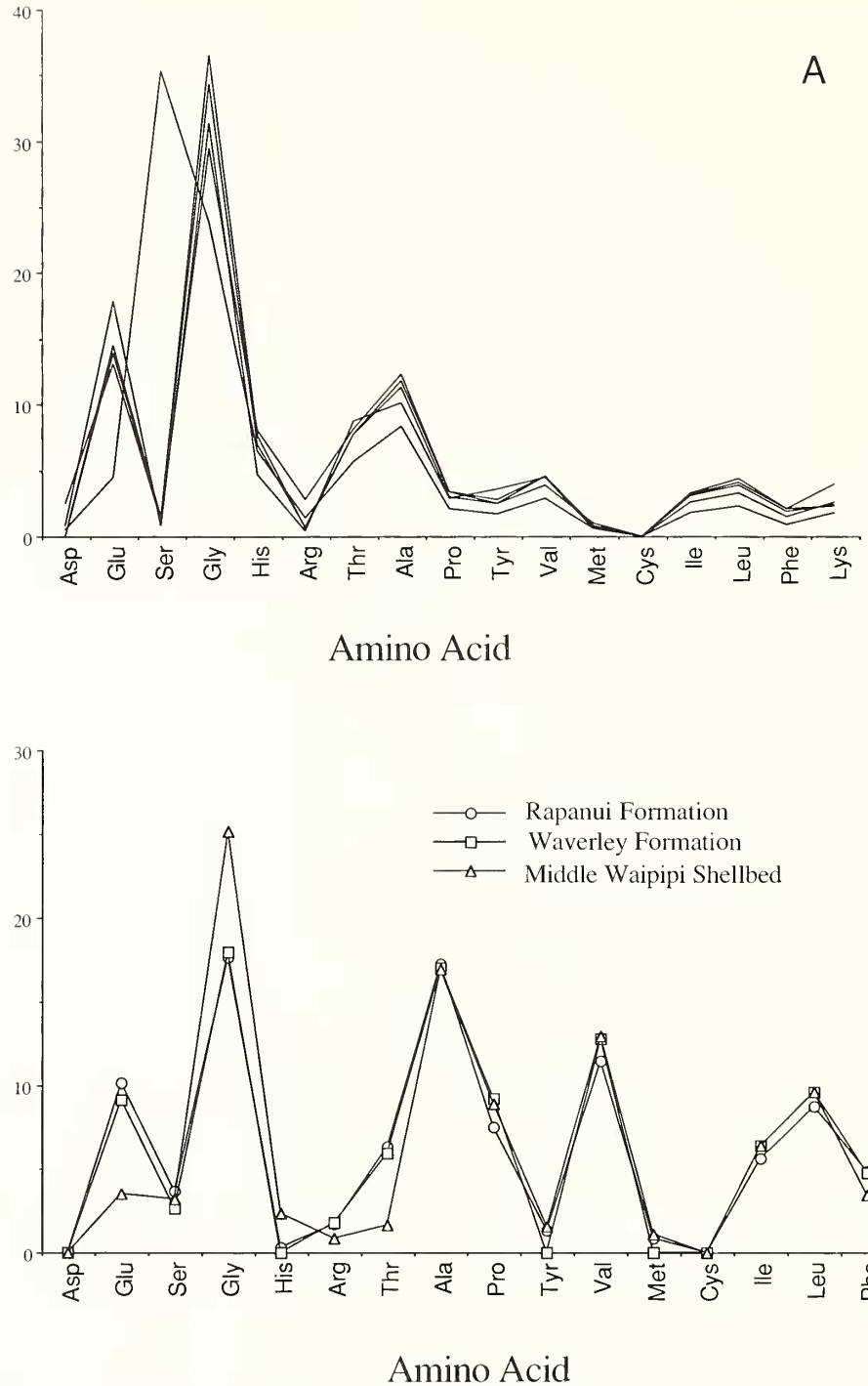
TEXT-FIG. 1. Pie charts showing the absolute proportions of selected amino acids (normalized to nmol/g) from the shells of Plio-Pleistocene *Neothyris* brachiopods, sediments (from the Plio-Pleistocene South Wanganui Basin of New Zealand; see Text-figure 3A for Formation details), and from finger tips (1 ml ≈ 1 g).

TABLE 1. Results of amino acid analyses (nmol/g). Figures in brackets refer to sample size.

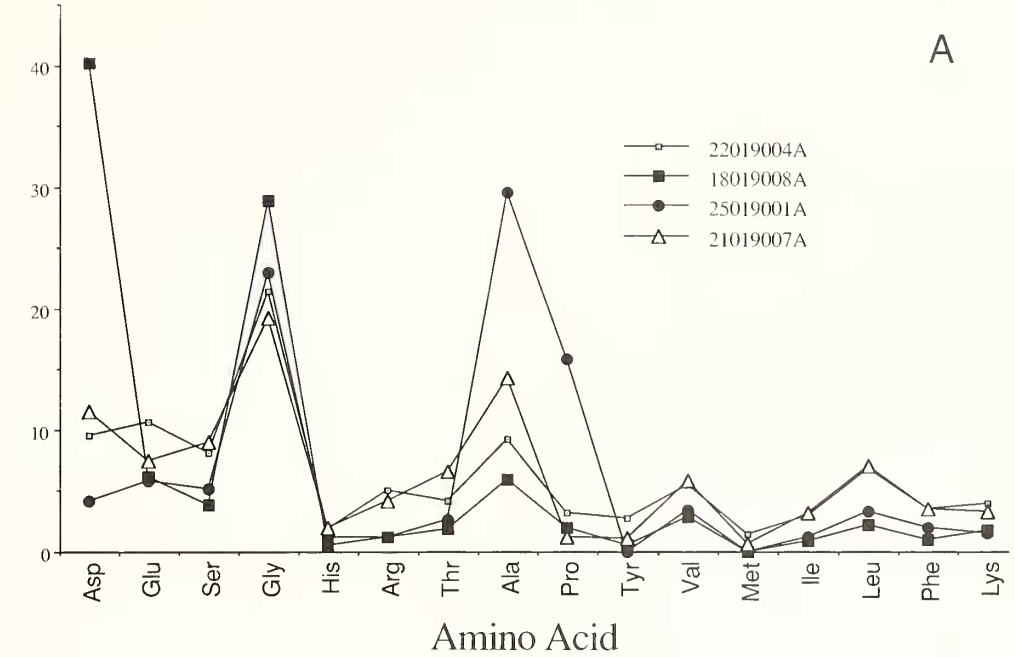
	Neothyris (4)		Finger-tips (8)		Sediments (3)	
	mean	S.D.	mean	S.D.	mean	S.D.
Aspartic acid	0·659	0·450	18·347	15·170	0·000	0·000
Glutamic acid	0·161	0·058	16·020	11·470	0·310	0·197
Serine	0·168	0·089	38·772	44·526	1·091	0·303
Glycine	0·505	0·143	56·211	19·670	5·978	0·768
Alanine	0·211	0·164	22·337	7·383	5·221	1·349
Valine	0·105	0·057	9·323	3·800	3·200	0·910

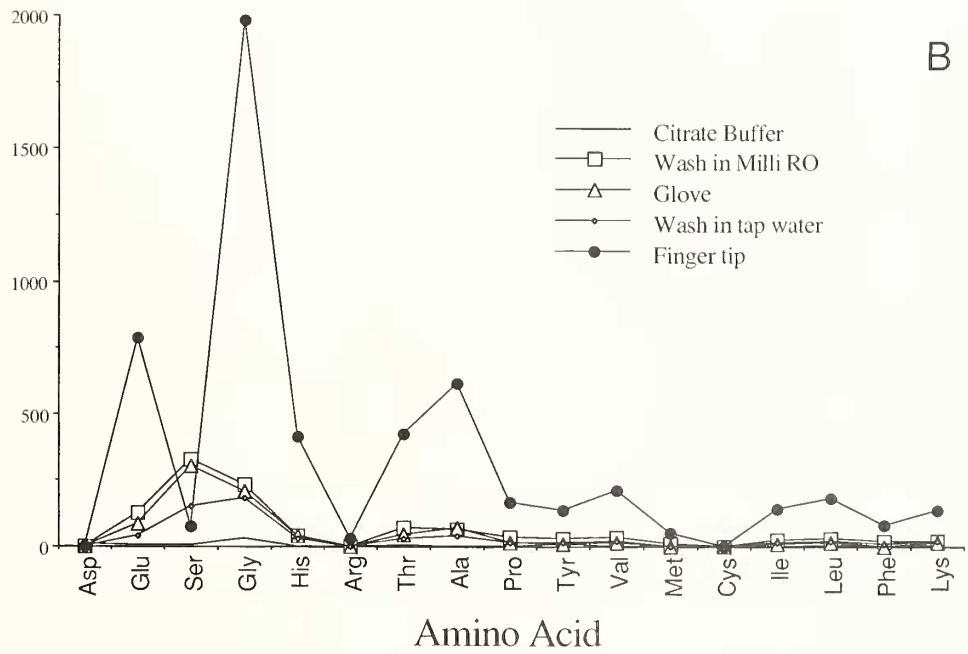
ABSOLUTE ABUNDANCE OF AMINO ACIDS

In absence terms, the quantities of amino acids that can be transmitted by finger-tips are considerably greater than those present in fossil shells and in sediments (Table 1; Text-fig. 1). Not surprisingly, there is slight variation in the yields for individual amino acids in some samples, but typically the levels for finger tips are 10 times higher than in sediments, and 100 times higher than in fossils (for example, glycine, see Table 1). Such levels are probably reasonably realistic assessments of the amounts of free amino acids which would be transferred to fossils or sediments by touching them with wet fingers. As the samples were not hydrolysed, amino acids still attached to one another in proteins from the skin would not have been quantified; small fragments of human skin are constantly being shed, and there is no doubt that the potential levels of amino acids transferred by finger contact would be greater than that measured here. Such contamination occurring at a critical stage of sample preparations will clearly overwhelm the indigenous amino acids.

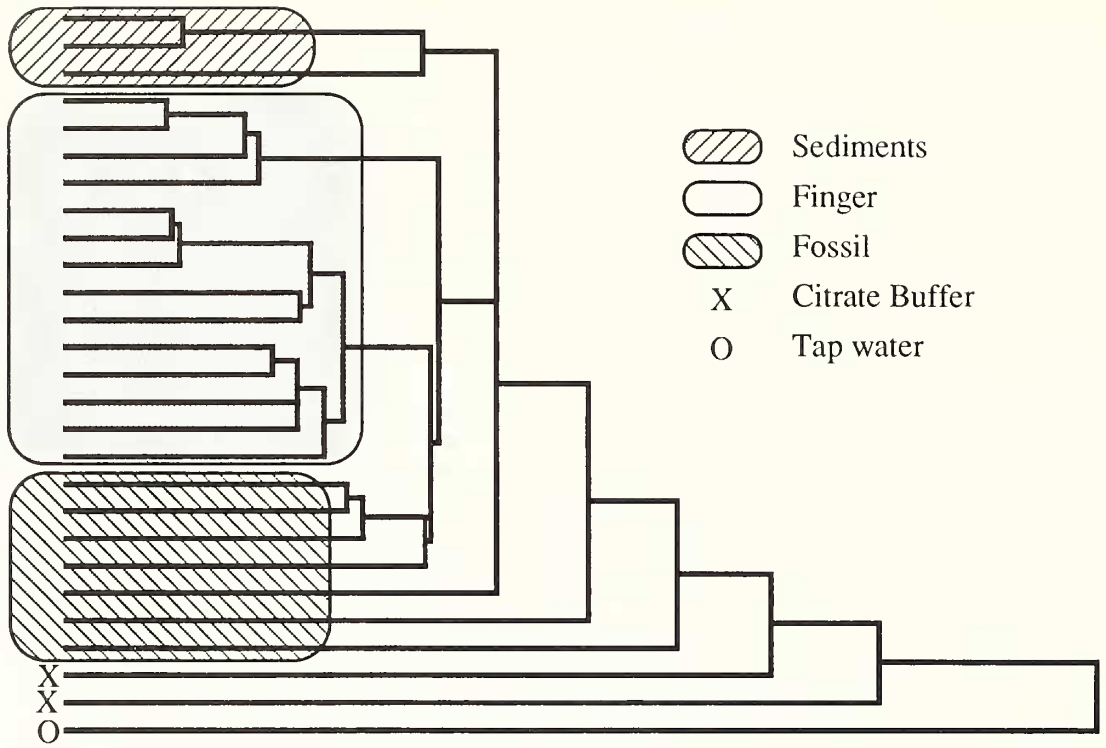


TEXT-FIG. 2. A, relative proportions (Mole %) of the common amino acids from the finger tips of five individuals from the first run of the experiment. B, relative proportion (Mole %) of the common amino acids for New Zealand sediments.





TEXT-FIG. 3. A, relative proportions (Mole %) of the amino acids from the shells of fossil *Neothyris*. Sample 25019001A shows the effect of incomplete removal of EDTA, a phenomenon which provides a check on the preparation purity of the sample. B, absolute properties (un-normalized, the concentrations (pmol) are for amino acids in 30 μl of buffer) showing the potential levels of contamination from latex gloves in comparison to finger tip data.



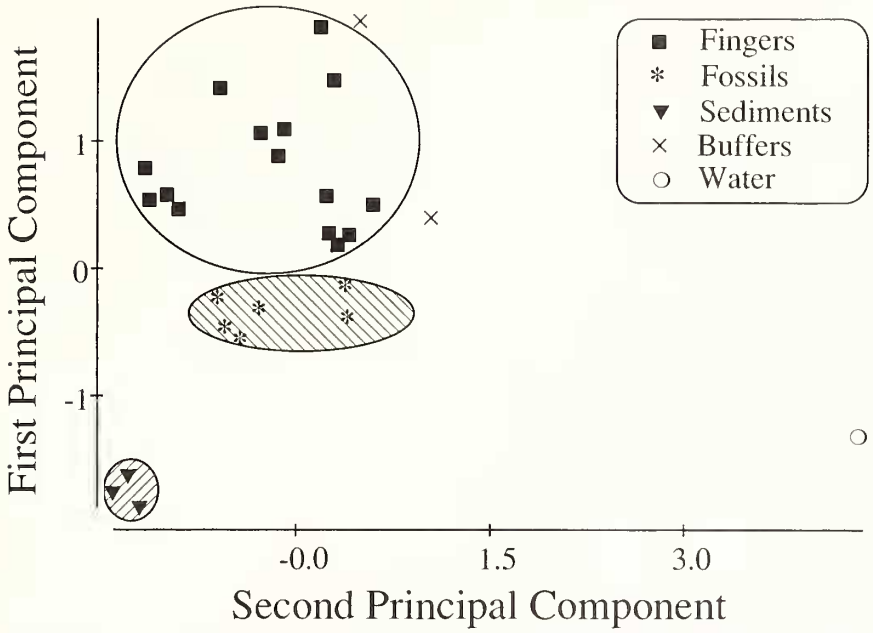
TEXT-FIG. 4. Single linkage cluster analysis (representing nearest neighbour groupings) for the relative proportion data (Mole %), generated by DATADESK™.

RELATIVE ABUNDANCE OF AMINO ACIDS

Amino acid data are often presented in the form of mole percentages which overcomes the difficulty of comparing samples of varying size. Text-figure 2A shows a remarkably consistent pattern in the relative abundance of finger-tip amino acids, with all individuals being rich in either serine or glycine. These results show some similarity with those of Oró and Skewes (1965), where serine is the most common amino acid. In sediments, serine and glycine are also the most abundant (Text-fig. 2B), while glycine is also a major constituent of fossil *Neothyris* shells (Text-fig. 3A). The relative proportions of amino acids are quite consistent within all three groups sampled (Text-figs 2A, 2B and 3A), to the extent that incomplete removal of EDTA from fossil samples becomes readily apparent (Text-fig. 3A). Apart from this sample, the fossil *Neothyris* all have consistent amino acid profiles.

Superficially there are a number of common features between these three datasets, but such subjective assessments are potentially misleading. To produce a more rigorous assessment of the relative similarity between samples, hierarchical clustering analysis using the statistical programme DATADESK™ on a Macintosh micro-computer was applied to the relative abundance data. The resulting cluster diagram resolves the finger tip, sediment and fossil shell amino acid data into discrete clusters (Text-fig. 4). The citrate buffer and tap water have been included as outgroups.

Principal component analysis (PCA) using DATADESK™ was also applied to the datasets (Text-fig. 5), and this confirms the groupings of the cluster plot. A useful attribute of PCA is that it is possible to use the eigen-vector values to determine which elements (in this case individual amino acids) are most important in distinguishing between the different groups. Text-figure 5 shows



TEXT-FIG. 5. Scatter plot of the first two principal components for relative proportion data (Mole %), generated by DATADESK™. These data do not take into account variations in the actual concentrations of the amino acids.

that the clear resolution between sediments, fossils, and fingers is achieved along the first principal component axis, which is aligned in the direction of maximum varibility and in this case contributes 26·9% of the total variation detected. Examining the eigen-vector values from this analysis, it is clear that this differentiation primarily reflects high scores for serine (+0·273), histidine (+0·276), arginine (−0·258), proline (−0·275), tyrosine (+0·229), valine (−0·377), methionine (−0·205), isoleucine (−0·323), leucine (−0·347) and phenylalanine (−0·345). The sign of these scores is also informative, indicating that samples which have high scores on this eigen-vector (i.e. the fingers) contain large amounts of serine, histidine and tyrosine, and relatively low yields of arginine, proline, valine, methionine, isoleucine, leucine and phenylalanine.

DISCUSSION

In one respect it is not surprising to discover a clear differentiation between these three groups of samples. Finger-tip amino acids are predominantly derived from human skin proteins. Amino acids from sediments presumably come from a complex mixture of sources including the breakdown of various life-forms which originally lived in or on the sediment and mobile amino acids carried in by percolating ground waters. The fossil amino acids considered here have been extracted from intracrystalline macromolecules; most published data from shells have also included a component of intercrystalline macromolecules (e.g. Jope 1967*a*, 1967*b*; Weiner *et al.* 1976), which are not protected by shell crystallites, and hence may be easily degraded or contaminated. It is very encouraging for the study of amino acids in these New Zealand successions that the finger-tip data can be so readily distinguished from the fossil and sediment data, both in terms of absolute abundance and mole percentages. Further samples of fossil shells have been analysed, and a similar pattern of differentiation from finger-tip and sediment data is apparent.

In practical terms, the use of clustering and statistical approaches represents an important and

rapid method of checking for human contamination in geological samples. As the absolute abundance of finger-tip amino acids is so much greater than that of fossils, any contamination should produce an analysis which clusters with the former rather than the latter, and this is probably also true for sediments although to a lesser extent.

The results of this investigation have also provided important information for sample preparation. High levels of contamination from finger-tip amino acids are to be expected, but these experiments have shown that even hands which have washed prior to handling samples could be a significant source of contamination. Much more surprising, and potentially worrying, is the discovery that even latex laboratory gloves can be a source of contamination amino acids (Text-fig. 3B). Latex gloves are widely used in laboratories, but obviously they give a false sense of security with modern high-sensitivity amino acid analysers capable of detecting a few picomoles (10^{-12} mole) of each amino acid. For optimum recovery of uncontaminated indigenous amino acids from fossils and sediments, it is necessary to minimize or completely eradicate any handling of the sample or of the interior surfaces of any vessel involved in the release of the incarcerated amino acids.

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